

Two New Species of *Diplobryum* (Podostemaceae, Podostemoideae) from Laos

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Two new species of *Diplobryum* are described from Laos, bringing the number of species in the genus to four. *Diplobryum vientianense* (n. sp.) resembles *D. minutale* in the foliose root and longitudinally split spathe. *Diplobryum koyamae* (n. sp.) is similar to *D. ramosum* in the long, ramified, flower-bearing, floating root. In root features *D. koyamae* is also similar to some species of *Polypleurum*. The phylogeny of the diversified genus *Diplobryum* remains uncertain. Recent collections and a comparison of Podostemaceae in Thailand and Laos indicate the necessity of further field research to better understand the flora and taxonomy of the family of Laos. A list of the species of Laos Podostemaceae is provided.

Key words: *Diplobryum koyamae*, *Diplobryum vientianense*, flora, Laos, Podostemaceae

Diplobryum, a member of subfamily Podostemoideae of the aquatic angiosperm family Podostemaceae, was established by Cusset (1972) for *D. minutale* C. Cusset from southern Vietnam. The species is characterized by its foliose root, capitate stigma, 20-ribbed capsule, the pedicel (= ovary stalk) protruding beyond the spathe and bracts, and the ellipsoid or lenticular seed (Cusset 1972, 1973). In the foliose root it is very similar to *Hydrobryum*. *Diplobryum ramosum*, the second species of *Diplobryum*, was described by Cusset (1992) from a specimen collected in northern Laos. It is distinct from *D. minutale* in its cylindrical, repeatedly branched, floating root and the linear, elongate stigma. The enlarged *Diplobryum*, composed of these two distinct species, is now defined only by the 20-ribbed capsule (Cusset 1992). However, because this character is seen elsewhere in *Hydrobryum* (M. Kato, unpubl. data), the question of recognizing *Diplobryum* as a distinct genus remains open. It will be able to be solved by a molecular phylogenetic analysis.

During field research in Laos in 1993 and 1994,

N. Fukuoka and H. Koyama collected four species of Podostemaceae, including one of *Polypleurum* and three of *Diplobryum* – *D. ramosum* and the other two undescribed. In this paper we describe the two as new and discuss our current understanding of the Podostemaceae in Laos and the systematic treatment of *Diplobryum*.

***Diplobryum vientianense* M. Kato & Fukuoka, sp. nov.** (Figs. 1, 2)

D. minutalis radicibus foliosis, bracteis 4-6, spatheis longitudinaliter fissilibus, capsulis 20-costatis simile, sed tepalis, staminibus et ovariiis duplo longioribus, stigmatibus linearibus, pedicellis fructuum plerumque flexis differt.

Type: LAOS. Vientiane Prov., Khu Kanna Nam Tok (Khu Kanna waterfall), 250 m alt., on rock in river bed; Jan 1, 1994, N. Fukuoka & H. Koyama L-65098 (holotype TNS; isotype L).

Plants fruiting and flowering (vegetative shoots

Key to the species of Diplobryum

1. Root foliose, adherent to rock; bracts 6, ovate-elliptic; spathella split longitudinally 2
 2. Ovary ca. 1 mm long; stigma capitate; stalk of capsule straight; seed coat with longitudinal ribs *D. minutale*
 2. Ovary ca. 2 mm long; stigma linear; stalk of capsule often bent; seed coat smooth *D. vientianense*
1. Root (axis) cylindrical, many times branched, floating; bracts 4-7, elliptic or deltoid-lanceolate; spathella ruptured near tip 3
 3. Flowers often at branching points of roots, nearly sessile; bracts imbricate, basal ones linear, caducous, upper ones smaller, hood-shaped *D. ramosum*
 3. Flowers usually alternate on both sides of root independently of branching; floral shoot 4-7(-10) mm long; lower bracts apart, less than 1 mm long, deltoid-oblong, persistent, upper ones imbricate, to 5 mm long, deltoid-lanceolate, acuminate at apex *D. koyamae*

not seen). Root foliose, irregularly lobed, with rhizoids in pads on ventral surface. Flowers densely arranged on dorsal surface of root, solitary, appressed; bracts 4-6, uniform but basal ones smaller, ovate, hood-shaped, obtuse at apex, 1.5-2 mm long, papillate; flower bud covered by spathella, spathella longitudinally split at anthesis, persistent, 2-2.5 mm long, elliptic, obtuse at apex, papillate except in center; stalk of ovary to 2 mm long; tepals borne below middle of stalk, 2 on each side of stamen, linear, 2-3 mm long; stamens 2, biforked 1/4-1/5 from tip, 4-5 mm long, much longer than pistil; anther ellipsoid, ca. 0.5 mm long; ovary 2-locular, ellipsoid, flattened, ca. 2 mm long, ca. 1 mm wide, protruding from spathella; stigmas 2, forked above base, equal, 0.6-1 mm long, linear, entire; ovules 22-27 per locule, borne over septa except in lower central small area; stalk of capsule usually bent when ripening, 2-2.5 mm long; capsule ellipsoid, flattened, 2-2.3 mm long, 1-1.2 mm wide, (16-)18-20-ribbed, 2-valved; seed ellipsoid, smooth.

Note: *Diplobryum vientianense* is most similar to *D. minutale* in having roots foliose (Fig. 1), bracts four to six, spathellas longitudinally split at anthesis, flowers protruding from the spathellas, and capsules about 20-ribbed (Fig. 2), but differs in having larger flowers (tepals, stamens and ovaries), linear stigmas, and the stalk of the capsule usually bent (Fig. 2). The two

species are distributed allopatrically: *D. vientianense* occurs in northern Laos and *D. minutale* in southern Vietnam. The phylogenetic relationship between the two species of *Diplobryum* and *Hydrobryum* with similar foliose roots remains uncertain (see also the note under *D. koyamae*).

***Diplobryum koyamae* M. Kato & Fukuoka, sp. nov.**
(Figs. 3, 4)

D. ramosi radicibus repetite ramosis, stigmatibus longis, linearibus, capsulis 18-20-costatis simile, sed bracteis usque ad 7, inferioribus redactis, remotis, superioribus imbricatis, acuminatis, usque ad 5 mm longis differt.

Type: Laos. Boli Kham Xai Prov., Tat Luek Nam Tok (Tat Luek waterfall), 260 m alt., Jan 3, 1994, *N. Fukuoka & H. Koyama* L-65169 (holotype TNS; isotypes L, MA, SHO).

Root (axis) ca. 50 cm long, cylindrical, 0.6-1.5 mm thick, floating except for base attached to rocks, repeatedly monopodially branched, with main axis slightly meandering. Flowering shoots alternate along length of roots, 4-7 mm long, rarely longer (below flower), with flowers apically and sometimes with 1 or 2 additional lateral flowers (shoot then ca. 10 mm long). Leaves (bracts) variable in number and size, 4-

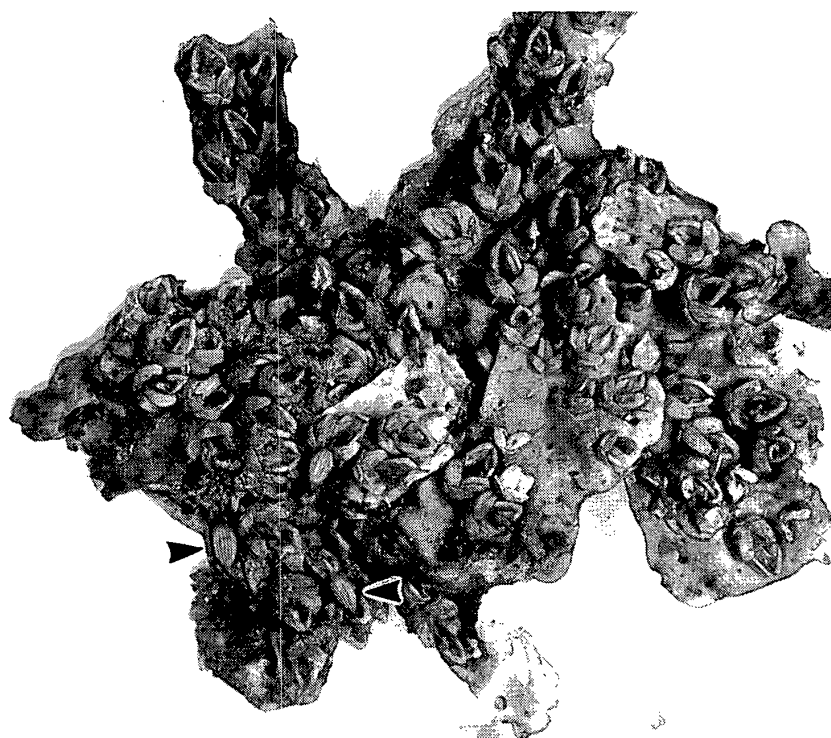


FIG. 1. *Diplobryum vientianense*. Holotype (N. Fukuoka & T. Koyama L-65098, TNS). Arrowheads indicate capsules, and most other capsules are lost. Scale = 1mm

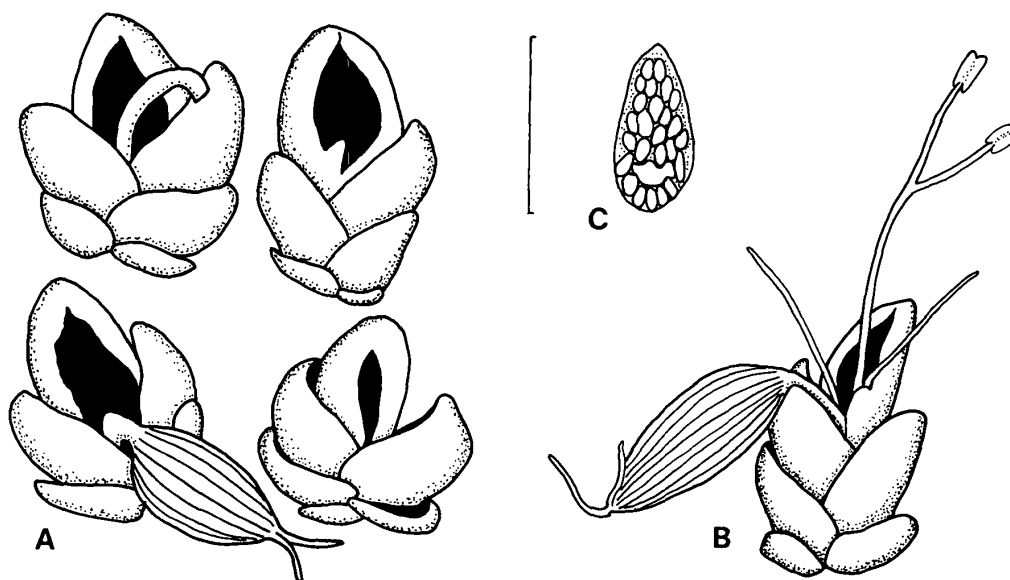


FIG. 2. *Diplobryum vientianense*. A. Four fruiting shoots appressed to root surface (three of four capsules are lost). Note persistent and longitudinally split spathe-like structures and bent capsule stalks. B. Flower with a pair of tepals, two stamens forked ca. 1/4 from tip, and ribbed ovaries on bent stalk. C. Ovules on septa except in sterile area in lower center. Bar = 2 mm.

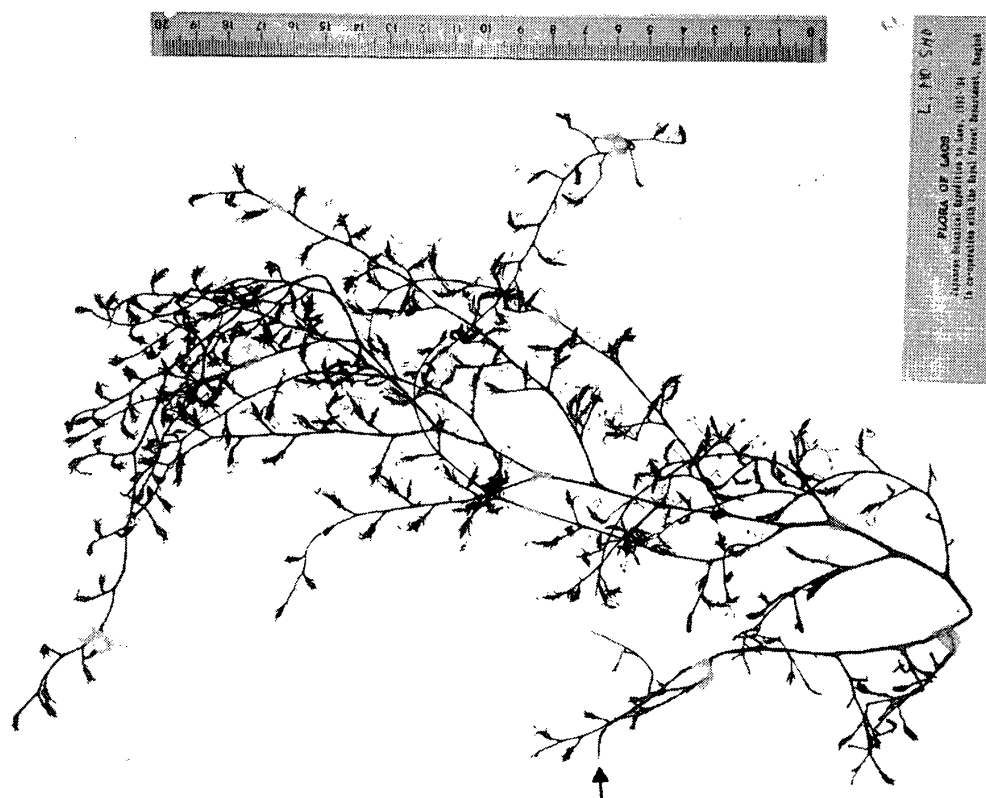


FIG. 3. *Diplobryum koyamae*. Holotype (N. Fukuoka & T. Koyama L-65169, TNS). Arrow indicates the proximal end of a floating root (axis).

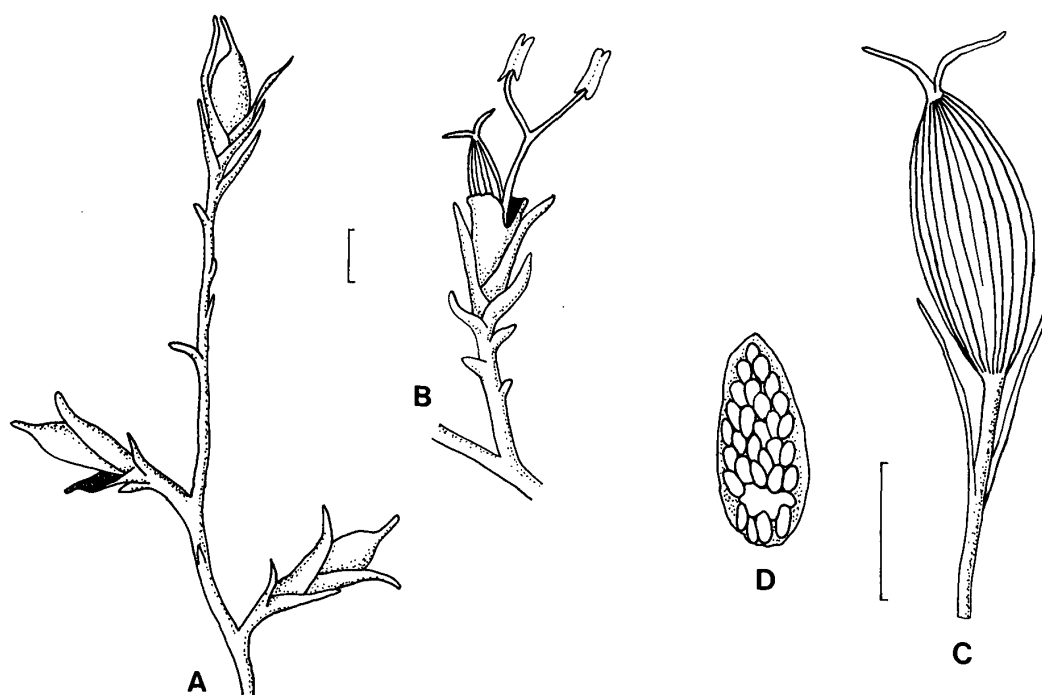


FIG. 4. *Diplobryum koyamae*. A. Three flower buds covered by mucronate spatheas, on leafy branches. B. Flower on branch. C. Ribbed ovary on stalk with two tepals. Stamen removed. D. Seeds on septa except in sterile area in lower center. Bars = 2 mm.

7, lower ones apart, reduced, less than 1 mm long, deltoid-oblong, persistent, upper ones ca. 5 mm long, embracing axis, membranaceous, deltoid-lanceolate, acuminate, sometimes elongate needle-like, imbricate. Spathella enclosing flower bud, narrowly elliptic, mucronate, mucro ca. 1.5 mm long, ruptured irregularly near tip at anthesis, subpersistent. Tepals 2 on each side of base of single filament, near middle of stalk of ovary, linear, 3-4 mm long. Stamens 2, arising with tepals, 6-10(-13) mm long, much longer than ovary, biforked 1/3 to 1/2 way from apex; anther oblong, 1.5-2 mm long, fixed at middle. Ovary on stalk 2-4 mm long, basal 1/3 to 1/2 enclosed by spathella, narrowly ellipsoid, flattened, 3.5-4.5 mm long, 1.2-1.5 mm wide, 2-locular; ovules borne over septa except in small lower central area, 25-35 per locule; stigmas 2, forked near base, linear, entire, 1-1.5 mm long. Capsule (fruit) narrowly ellipsoid, flattened, as large as or slightly larger than ovary, 18-20-ribbed, pedicellate, pedicel ca. 4 mm long; seed ellipsoid, smooth.

Note: *Diplobryum koyamae* is most similar, and probably closely related, to *D. ramosum* in the floating, branched root (Fig. 3), biforked stamen (filament) longer than the ovary, the long, linear stigma, and the capsule with about 20 ribs (Fig. 4). There are some differences in these species. In *D. koyamae* the flowering shoot is long and arranged along the length of the root independently of root branching, the bracts range from 4 to 7, with lower ones less than 1 mm long, separate and persistent, and the upper ones deltoid-lanceolate, acuminate and up to 5 mm long (Fig. 4). In *D. ramosum* the flowers are often located at the points of root ramification, the bracts imbricate, the basal ones linear, caducous, the upper ones smaller than the basals, hood-shaped (Cusset 1992). The organographic relationship of the root and flowering shoot is unclear and it is also unclear if the floating axis of *D. koyamae* is homologous with the root. In root morphology and habit, *D. koyamae* and *D. ramosum* are distinct from the foliar-rooting *D. minutale* and *D. vientianense*.

Although *Diplobryum vientianense* and *D. koyamae* seem to be closely related *D. minutale* and *D. ramosum*, respectively, the phylogenetic relationships between these two species groups remain uncertain. Although the four species of *Diplobryum* share the approximately 20-ribbed capsules, they are more similar in habit to species of *Hydrobryum* and *Polypleurum* than they are to each other. *Diplobryum minutale* and *D. vientianense* are very similar to species of *Hydrobryum* and also to *Hanseniella* in the foliose, adhering roots. Capsules with about 20 ribs are also seen in species of *Hydrobryum* (e.g. *H. bifoliatum* C. Cusset; M. Kato, unpubl. data), which generally has capsules with 12-16 ribs. *Diplobryum koyamae* and *D. ramosum* resemble species of *Polypleurum*, particularly *P. elongatum* (Gardn.) J. B. Hall, in the floating, ramified, markedly long (up to 50 cm) root with flowers along the length (Cusset 1992, Mathew & Satheesh 1997), but distinctly differs from *Polypleurum* in the number of ribs on the capsule (20 vs. 8). Generally, the root of Podostemaceae is subcylindrical, ribbon-like or foliose, creeping and adherent to rocks along the length by adhesive root hairs. *Diplobryum koyamae*, *D. ramosum* and some species of *Polypleurum* are exceptions in the floating habit of the root. Whether the floating roots of *Diplobryum* and *Polypleurum* are the result of parallelism or convergence remains to be investigated.

Cusset (1972, 1973, 1992) and Cusset & Cusset (1988) recorded three genera and four species in two subfamilies (families in their treatment) from Laos: *Cladopus pierrei* (Lecomte) C. Cusset and *Diplobryum ramosum*, Podostemoideae, and *Dalzellia carinata* (Lecomte) C. Cusset and *D. diversifolia* (Lecomte) C. Cusset, Tristichioideae. During a brief trip to Laos N. Fukuoka and H. Koyama collected *Polypleurum wallichii* (R. Br. ex Griff.) Warm., *Diplobryum koyamae*, *D. ramosum*, and *D. vientianense*, bringing the number of genera to four and the number of species to seven in two subfamilies (Table 1). *Polypleurum wallichii* is a new record for Laos, and the Fukuoka and Koyama specimen of *D. ramo-*

TABLE 1. A list of species of Podostemaceae of Laos.

Species	Collection*
Podostemoideae	
<i>Cladopus pierrei</i>	Campassak, Bassac, <i>F.(A.)F.J. Harmand s.n.</i> (as <i>Pierre 5194</i>) (holotype P)
<i>Diplobryum koyamae</i>	Boli Kham Xai, Tat Luek waterfall, <i>N. Fukuoka & T. Koyama L-65169</i> (holotype TNS; isotypes L, MO, SHO)
<i>Diplobryum ramosum</i>	N Vientiane, Phu Khao Khwai, <i>T. Smitinand s.n.</i> (holotype C) Vientiane, Mt. Phu Kao Kwai, <i>N. Fukuoka & T. Koyama L-65040</i> (L, MO, SHO, TI, TNS)
<i>Diplobryum vientianense</i>	Vientiane, Khu Kanna waterfall, <i>N. Fukuoka & H. Koyama L-65098</i> (holotype TNS, isotype L)
<i>Polylepium wallichii</i>	Vientiane, Khu Kanna waterfall, <i>N. Fukuoka & T. Koyama L-65097</i> (TNS)
Tristichioideae	
<i>Dalzellia carinata</i>	Sedone, Attopeu, <i>F.(A.)F.J. Harmand s.n.</i> (P) Campassak, Bassac, <i>F.(A.)F.J. Harmand s.n.</i> (as <i>Pierre 2313</i>) p.p. (P)
<i>Dalzellia diversifolia</i>	Sedone, Attopeu, <i>F.(A.)F.J. Harmand s.n.</i> (P) Campassak, Bassac, <i>F.(A.)F.J. Harmand s.n.</i> (as <i>Pierre 2313</i>) p.p. (P)

* From Cusset (1972, 1973, 1992) and specimens deposited in TNS and SHO. Herbarium abbreviations except for SHO follow Holmgren *et al.* (1990); SHO is used for Shoei Junior College Herbarium.

sum is the second collection from the type locality. In comparison, two subfamilies and 10 species have been recorded from Thailand (Cusset 1992), but recent field research led to the discovery of about 10 additional undescribed species of the subfamilies, indicating that about 20 species occur in Thailand (M. Kato, unpubl. data). It is possible that Laos, which is adjacent to Thailand, contains many more species than known at present and further extensive field research is required to locate them.

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